C·R·E·S®
CENTER FOR REPRODUCTION OF ENDANGERED SPECIES

REPORT

SUMMER 1996

MAY 1 1996
SAN DIEGO ZOO
LIBRARY

A Team Effort: Carnivore Preservation

At the beginning of 1995, the equipment "wish list" for the reproductive physiology division of CRES read: 1 stereo microscope, 2 vacuum pumps, 1 diluter/dispenser, and 1 small incubator. However, it took the support of the local veterinary community, as well as responsible pet owners who brought in their dogs to be neutered or spayed, for this project to be possible. Endangered carnivores such as cheetahs, Cape hunting dogs, tigers, and fishing cats are just a few of the species to benefit from canid reproduction studies at CRES.

The preservation of carnivores is of critical importance because of the role predators play in maintaining a balance between herbivore populations and the vegetation consumed by those herbivores. As carnivore populations dwindle in the wild, captive-breeding programs will assume an increasingly significant component of conservation strategies. Germ plasm cryopreservation will serve as a vital resource for captive breeding as it will minimize the space and costs required for holding large numbers of individuals. Germ plasm—ova, sperm, and embryos—can be stored in liquid nitrogen until it is needed to enhance the genetic diversity of captive or wild populations.

Sperm production is continuous throughout life in male mammals. Therefore, it is possible to extract mature sperm from an animal at any time after sexual maturity. The reproductive physiology division of CRES currently harvests testicular sperm postmortem from many endangered species and good quality samples are cryopreserved, thus preserving potential genetic contributions from deceased individuals.

Female mammals, unlike males, do not continue to generate gametes after they are born. Thus, at birth a female's ovaries contain a reservoir of immature

Continued on page 2

Polar bears are just one of the species to benefit from CRES studies on carnivore reproduction.

CRES® is operated by the Zoological Society of San Diego.

Saving Rhino Species
Captive reproduction is the key. See page 3.

The Society's New Web Site
Come on a "virtual visit"! See page 5.

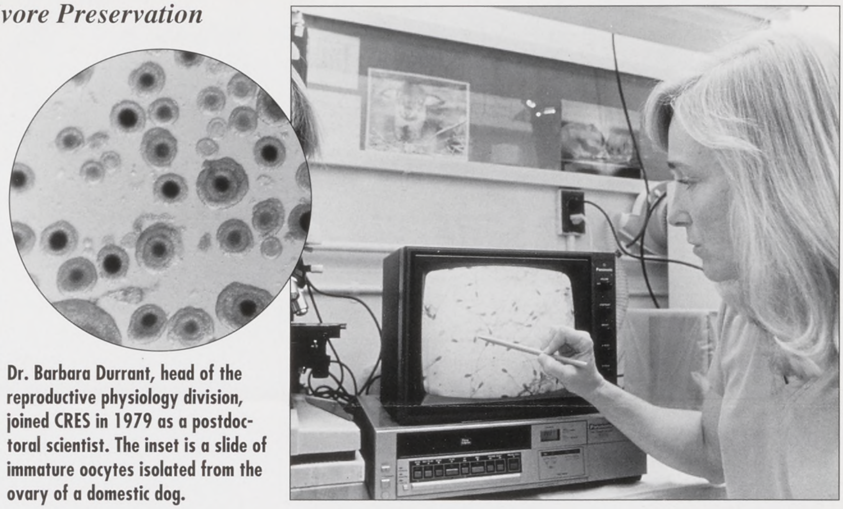
Estate Planning Techniques
How you can help CRES. See back cover.

A Team Effort: Carnivore Preservation

Continued from page 1

oocytes sufficient to last the lifetime of the animal. Under the influence of reproductive hormones, a few immature oocytes each cycle begin to grow and mature in preparation for ovulation. Rescue of female gametes at necropsy has been less successful than collection of sperm, because the status of ova is much more dependent on sexual maturity, breeding season, and stage of the estrous cycle at the time of death than is sperm. Oocyte collections have been severely limited because so few females come to necropsy at the stage of the estrous cycle when mature oocytes can be recovered. Even if mature oocytes are present, they represent only a small fraction of the total number of oocytes within the ovary. The ability to remove immature oocytes from the ovary, then grow, mature, and fertilize them *in vitro,* will greatly enhance the preservation of genetic material from endangered species.

The domestic dog is used as a model for endangered carnivores because of its taxonomic, physiologic, and anatomical similarities to other canids (dogs), felids (cats), and ursids (bears). Canine ovaries and testes are donated by local veterinary clinics and animal shelters following spay and neuter for population control. This generous response from the local veterinary community is gratefully acknowledged.



Dr. Barbara Durrant, head of the reproductive physiology division, joined CRES in 1979 as a postdoctoral scientist. The inset is a slide of immature oocytes isolated from the ovary of a domestic dog.

1996 "Wish List" for Reproductive Physiology

Liquid nitrogen freezer - $10,000. This is needed to store oocytes and sperm from carnivore species such as the cheetah, polar bear, fishing cat, tiger, and maned wolf.

Power Macintosh 9500 computer - $5,000. Needed for the storage and analysis of data generated by this and other reproductive physiology division projects.

The development of optimal techniques of *in vitro* growth, maturation, and fertilization for oocytes of carnivore species will require several years of investgation and will include the following studies:

- isolation of immature oocytes from whole ovaries or ovarian biopsies
- *in vitro* growth and maturation of ovarian oocytes
- cryopreservation of testicular sperm
- *in vitro* fertilization of *in vitro* matured oocytes
- embryo development of *in vitro* fertilized oocytes
- cryopreservation of ovarian oocytes before or after *in vitro* maturation.

This ambitious project could not be completed without the assistance of our many donors. The involvement of internal and external funding agencies, local businesses, and individuals has made the project a reality, and we sincerely appreciate their support.

The Morris Animal Foundation, in a three-year grant, provides one-half of the salary of a postdoctoral fellow for full-time work on the carnivore project. Supplies for the project are also purchased with funds from this grant.

The stereo microscope needed to identify and classify immature oocytes was purchased with grant funds from the Armstrong-McDonald Foundation. In addition, the remaining half of the postdoctoral fellow salary and project supplies are provided by the grant. This foundation has contributed to reproductive physiology research at CRES since 1979 and has truly been the cornerstone of extramural funding for this division.

The L.J. Skaggs and Mary C. Skaggs Foundation has supported several reproductive physiology projects over the last ten years. Most recently, a grant from this foundation was used to purchase a diluter/dispenser, which streamlines the synthesis of various media needed for the carnivore oocyte project.

The Bayer Corporation, Pharmaceutical Division, made a significant donation of equipment to CRES in 1995, including two vacuum pumps and the small incubator needed by reproductive physiology. These items are used in the preparation of oocytes and sperm prior to *in vitro* culture.

As you can see, the carnivore oocyte project is a team effort. The progress made is due to the interest and support of a diverse group of people and organizations. The common goal of everyone involved is the preservation of some of the world's most fascinating animals.

—Barbara Durrant, Ph.D.,
Head of Reproductive
Physiology Division/CRES

Wishing for Rhino Reproduction

The endocrinology and behavior divisions at CRES have a major item on their 1996 "wish list": a three-quarter-ton, 4-wheel-drive truck to be used at the Wild Animal Park for fertility studies on the northern white and southern white rhinos.

All species of rhinoceros are endangered. Wild populations of rhinos are being annihilated by poaching and loss of habitat. The critical status of these populations demands the success of captive reproduction in order to secure self-sustaining populations for the future. The San Diego Wild Animal Park maintains four species of rhinoceros: black, southern white, northern white, and Indian. All but the northern white rhino species have reproduced at the Park.

While this is remarkable and one of the best records in any captive facility, there are still major reproductive problems to overcome in order to have self-sustaining populations. For example, the Park has had the amazing success of more than 80 births of southern white rhinos—however, only animals imported from the wild have reproduced. None of the offspring born in captivity (i.e., F1 generation) have reproduced; in fact, throughout North America, only 2 of 50 F1 animals have reproduced. This is obviously a serious problem because the wild-born population is advancing in age. We must begin to understand the reproductive problems in the F1 generation if we are to continue a viable captive population of this species.

Another species, the northern white rhino, is on the verge of extinction: only one population of 30 animals exists in the wild, and only 11 are held in captivity. The Park has the only population of this species in North America, with two males and two females. No matings had occurred in this species at the Park until last year. Our ability to monitor reproductive cycles and diagnose pregnancy is crucial to our goal of reproducing this rare species.

For the black rhino as well, the reproductive rate in the captive population is much lower than that which occurs in the wild. Again, this is another rhino species that demands a complete reproductive study.

The staff members in the endocrinology division at CRES have established and validated methodology to monitor reproduction in all three of these rhino species. Since blood samples cannot be routinely taken from the rhinos, we have developed fecal hormone assays to permit the evaluation of ovarian function and the detection of pregnancy. We have worked toward this goal during the last five years. Daily fecal samples are collected from selected animals from each of these species. Once collected, samples are processed to determine their hormonal content. In collaboration with behavior division staff members, who are recording observations of behavioral variables, we propose to correlate hormonal results to behavioral observations of the rhinos.

With this information, we will be able to determine whether individual animals are ovulating, infertile, or pregnant. Without hormonal analysis, it is currently impossible to make these determinations. Technical support in the laboratory and supply funds are needed to complete this study, which is critical for the worldwide northern white rhino population.

—Nancy Czekala, Endocrinology Specialist/CRES

With more than 80 southern white rhino births since the early 1970s, this is one of the great success stories for the San Diego Wild Animal Park.

INSIDE CRES

Genetics

Typically, animals receive one copy each of their genes from their father and another from the mother. When the parental copies of genes differ, it is possible to characterize the descent of genes through a family tree or pedigree. Paternity analysis is a specialized use of this type of information. Previously, we developed a noninvasive method of paternity analysis in giant pandas when Dr. Ya-ping Zhang from the Kunming Institute of Zoology in China was a visiting scientist at CRES. More recently, we have utilized the analysis of variable DNA sequences called microsatellites, which are typically used in human genetic studies, to investigate paternity in gorilla groups living in zoos. The encouraging trend toward keeping animals in more natural social groupings may lead to unknown paternity of offspring born into a group with more than one resident sexually mature male. Fortunately, noninvasive genetic studies—involving collecting shed hair from the mother, offspring, and all males who possibly could be the father—are able to resolve which male is the sire of each offspring. Accurate assignment of parentage is an essential feature of conservation management of small populations, such as those held in zoos. We have studied the lowland gorilla groups at the Los Angeles and Lincoln Park (Chicago) zoos in order to help curators and population managers confirm pedigree assignments. Interestingly, the dominant male gorilla, known as a silverback, was indeed the sire of most of the offspring in each group. However, in both zoos, this dominant male was not the sire of all offspring born into the group.

Virology

Immunodeficiency lentiviruses are retroviruses that cause a chronic infection that, in many individuals, results in suppression of the immune system and development of opportunistic infections. Found in humans, nonhuman primates, several species of cats, and domestic cattle, some members of this group of viruses affect endangered species that are the focus of intense captive management programs. Dr. Mike Worley and Ann Misuraca of the virology division of CRES, in collaboration with Dr. Tom Phillips of The Scripps Research Institute and veterinarians at the San Diego Wild Animal Park, are studying various aspects of feline immunodeficiency virus (FIV) in cheetahs. Cheetahs seem to be more susceptible to infectious diseases and experience a greater incidence of wasting than other species of nondomestic cats. The movement of infected animals between zoological institutions is an issue of utmost concern to the cheetah Species Survival Plan (SSP). The research at CRES is focusing on the development of specific and sensitive diagnostic tests that can rapidly identify infected animals. Specific protocols can then be established for the separate management of the known infected individuals.

CRES Grants

The Center for Reproduction of Endangered Species is grateful to the following for their support of imperiled wildlife:

An ***Alice C. Tyler Perpetual Trust*** *Environmental Grant of $30,000 has been made in support of the Giant Panda Conservation Program. The grant will provide training for a Chinese scientist in genetics at CRES under the direction of Dr. Oliver Ryder. In the endocrinology division, a laboratory technician will examine the chemical composition of scent marks and changes in the panda's hormonal and reproductive state.*

The ***John and Beverly Stauffer Foundation*** *has made a grant to continue Dr. Oliver Ryder's vital work in black rhinoceros conservation. Dr. Ryder will incorporate state-of-the-art methods of analysis of DNA variation in order to further examine the genetic differentiation of black rhino populations in eastern and southern Africa.*

These individuals have made equipment gifts to CRES or are supporting CRES projects:

Jeanette Henderson *has purchased an inverted microscope system that can be used by several divisions within CRES.* ***Beverly Muchnic*** *has made a donation to the CRES endowment fund, which is vital to the future of CRES.* ***John H. and Margaret F. Scott*** *have provided funds to assist with the CRES peccary research project in Paraguay.*

A Wildly Wonderful Animal Collection Benefits CRES

A breathtaking new collection of ornaments from Silvestri® has been created in support of CRES and its efforts to find vital alternatives to the continuing loss of wildlife. As part of the Zoological Society of San Diego, CRES represents a worldwide conservation and research effort. Through CRES projects, wildlife and scientific experts are finding ways to help rare animals reproduce that can no longer survive on their own in the wild.

Silvestri's selection of these rare species includes the cheetah, gorilla, Siberian tiger, African lion, African elephant, polar bear, Bactrian camel, ostrich, koala, slow loris, douc langur, black rhino, Sichuan takin, black-necked swan, and meerkat, all depicted in their natural habitats in amazing, accurate detail. They are now on display in the gift shops at the Zoo and the Wild Animal Park. A portion of the proceeds from the sale of these ornaments will benefit the important work currently under way at CRES.

Illustration by Bill Noonan

Visit a Wild Web Site!

*The next best thing to a visit to the San Diego Zoo or the San Diego Wild Animal Park is a "virtual visit" to the Zoological Society's Web site. You can now find us at this Web address—***http./www.sandiegozoo.org***—and the click of a mouse will send you to the Zoo, Wild Animal Park, or CRES sections. So log on to the World Wide Web and see where our conservation projects are leading us.*

This Galápagos tortoise Geochelone elephantopus, hatching at the San Diego Zoo's Klauber-Shaw Reptile House in May of 1994, was the first since 1988. These large and long-lived tortoises from the Galápagos Archipelago off the coast of South America are considered endangered.

September 1996

The Galápagos tortoise is a browser that eats the leaves and fruits of a variety of plants.

SUNDAY	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY
1	2 Labor Day	3	4	5	6	7 White Elephant Sale Begins
8 Grandparents Day	9	10	11	12	13	14 Rosh Hashanah
15 White Elephant Sale Ends	16	17	18	19	20	21 Celebration For the Critters
22 Kol Nidre Fall Equinox	23 Yom Kippur	24	25	26 Lunar Ec		28
29	30				AUGUST	OCTOBER

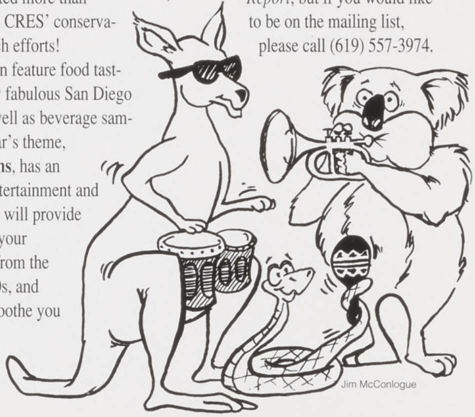
Save the Date for Celebration!

Mark your calendars and tell your friends to join you at the "wildest" party in town! The 16th annual Celebration for the Critters will be held at the San Diego Zoo on Saturday, September 21, from 7 P.M. to midnight. This very festive fund-raiser benefits the Zoological Society's Center for Reproduction of Endangered Species (CRES). Last year's event was a big success and netted more than $80,000 toward CRES' conservation and research efforts!

We will again feature food tastings from many fabulous San Diego restaurants as well as beverage samplings. This year's theme, **Jungle Rhythms**, has an emphasis on entertainment and music. Rockola will provide the rhythms of your favorite songs from the 1960s and 1970s, and Interplay will soothe you with jazz rhythms.

Tropical rhythms will also echo through the night with our Calypso trio. So . . . don't miss a beat—come join the fun and mingle with the Zoo's furry, feathered, and scaly party animals!

Tickets are $40 per person ($50 after September 11, 1996) and will go on sale this summer. Additional information will be in the next issue of *CRES Report*, but if you would like to be on the mailing list, please call (619) 557-3974.



Jim McConlogue

Planned Giving Can Benefit CRES

You can help CRES and yourself in many different ways. By using planned giving techniques, you can significantly aid CRES in meeting its goals and secure many benefits for yourself in the process. Here are some choices that will provide a number of mutual advantages.

LIFE INCOME PLANS

A Charitable Remainder Trust is formidable sounding, perhaps, but simple in concept, and you can establish one. With this trust, you can irrevocably transfer cash, securities, or real estate into a C.R.T. and receive lifetime earnings checks. In addition, there are many tax advantages such as an immediate charitable gift income tax deduction, avoidance of capital gains taxes from the sale of appreciated property, and possible reduction or elimination of estate taxes. If you do not want to set up your own trust fund, you can utilize the Zoological Society's Pooled Income Fund, which provides essentially the same advantages as a personal trust but is as simple as opening a bank account.

LIFE INSURANCE

A very impressive future gift to CRES can be created through life insurance. Premiums paid are deductible if the Society owns the policy, which further reduces the already modest present cost of such a gift arrangement.

REAL ESTATE

Real property of many types can make great gifts with a low basis cost. And real estate can be transferred into charitable trusts to provide you lifetime income and tax advantages.

SECURITIES

Many types of securities, especially if highly appreciated, can make wonderful charitable gifts. You may deduct the full market value on the date of the gift, not just your basis, and thereby make a substantial immediate gift at a very low actual cost.

I.R.A.

You can make the Society the primary or contingent beneficiary of your I.R.A. and, thereby, possibly make a fine future gift to CRES with no actual additional cost above your planned I.R.A. contributions.

Brochures or other information regarding these giving opportunities are yours for the asking with absolutely no obligation. Just phone Patrick O'Reiley, the Society's planned giving director, at (619) 685-3215 and you will receive a prompt response.

CRES REPORT
CENTER FOR REPRODUCTION OF ENDANGERED SPECIES

EDITOR:
Mary Sekulovich

EDITORIAL ASSISTANT:
Betsy Sanders

SPECIAL THANKS TO CRES REPORT CONTRIBUTORS:
Nancy Czekala
Barbara Durrant, Ph.D.
Judy Kinsell
Joanne Leidenberger
Patrick O'Reiley
Amy Parrott
Oliver Ryder, Ph.D.
Michael Worley, D.V.M.

GRAPHIC DESIGN:
Warner Design Associates, Inc.

PHOTOGRAPHS/ ILLUSTRATIONS BY:
Ron Gordon Garrison
Ken Kelley
Jim McConlogue
Bill Noonan

SAN DIEGO ZOO · SAN DIEGO WILD ANIMAL PARK ·

© Zoological Society of San Diego, 1996.
All rights reserved.

Printed on recycled paper.

Zoological Society of San Diego
Development Department
Post Office Box 551
San Diego, California 92112

CRES REPORT
CENTER FOR REPRODUCTION OF ENDANGERED SPECIES

Non-Profit Organization
U.S. Postage
PAID
San Diego, California
Permit Number 410